FREEDOM OF INFORMATION SUMMARY

Original New Animal Drug Application

NADA 141-188

Marquis™
(15% w/w ponazuril)
Antiprotozoal Oral Paste

"...for the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona.*"

For Oral Use in Horses

Sponsored by:

Bayer Corporation
Agriculture Division
Animal Health

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I. GENERAL INFORMATION

NADA Number: 141-188

Sponsor: Bayer Corporation
Agriculture Division
Animal Health
P. O. Box 390
Shawnee Mission, Kansas 66201

Generic Name: ponazuril

Trade Name: Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste

Marketing Status: This is a prescription (Rx) product which carries the following caution statement: "Federal (USA) law restricts this drug to use by or on the order of a licensed veterinarian."

II. INDICATIONS FOR USE

Marquis (ponazuril) is indicated for the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona.*

III. DOSAGE FORM, ROUTE OF ADMINISTRATION, AND DOSAGE

Dosage Form: Marquis (ponazuril) is available in cartons containing four (4) syringe applicators and one (1) syringe plunger. Each syringe barrel of Marquis (ponazuril) will deliver 127 grams of paste. Each gram of paste contains 150 mg of ponazuril (15% w/w).

Route of Administration: Marquis (ponazuril) is administered orally.

Recommended Dosage: Each syringe barrel of Marquis (ponazuril) contains enough paste to treat one (1) 1,200 lb. (545 kg) horse for seven (7) days, at a dose rate of 5 mg/kg (2.27 mg/lb.) body weight. The plunger contains a dosage ring calibrated for a dose rate of 5 mg/kg (2.27 mg/lb.) body weight and marked for horse weights from 600 to 1,200 lb. (273 to 545 kg). The syringe barrels are packaged in units of four with one reusable plunger. This package provides sufficient paste to treat one 1,200 lb. (545 kg) horse for 28 days at a dose rate of 5 mg/kg (2.27 mg/lb.) body weight.

IV. EFFECTIVENESS

A. Dosage Characterization

To characterize the dose of ponazuril necessary to treat infections of *Sarcocystis neurona*, several lines of evidence were considered. Studies have demonstrated that ponazuril is an effective anticoccidial drug for the treatment of gastrointestinal and somatic coccidiosis in several species of mammals and birds, including cattle, sheep, swine, rabbits and chickens at doses of 7-20 mg/kg. Therefore, a series of studies was conducted to determine a dose of ponazuril that would be effective in horses against *Sarcocystis neurona.* First, an *in vitro* test was conducted to determine the dose of drug necessary in the cerebrospinal fluid of the horse. Second, a pharmacokinetic study defined the level of drug that could be obtained in both serum and CSF. Finally, two doses were tested in naturally infected horses in a clinical field study.

1. *In vitro* Susceptibility Study

a. Type of Study/Purpose: To evaluate the activity of ponazuril against the intracellular stages of *Sarcocystis neurona* in cell culture.

b. Investigators:

J. P. Dubey, Ph.D., USDA, Agricultural Research Service, Livestock and Poultry Sciences Institute, Parasite Biology and Epidemiology Laboratory, Beltsville, MD 20705

D. S. Lindsay, Ph.D., Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, 1410 Prices Fork Road, Blacksburg, VA 24061-0342

T. J. Kennedy, Ph.D., Bayer Corporation, Agriculture Division Animal Health, P.O. Box 390, Shawnee Mission, KS 66201

c. General Design: *In vitro* evaluation of effectiveness to inhibit merozoite development in cell cultures.

1) Test Media: Bovine turbinate or African green monkey kidney cells grown to confluence in supplemented RPMI media.

2) Treatments: 0.001, 0.01, 0.1, 1.0, 5.0 µg/mL of ponazuril in the culture media

3) Procedure: All cultures were grown in 25 cm^2 plastic cell culture flasks, incubated at 37°C in a reduced atmosphere and stored under the same conditions. The assay involved an assessment of merozoite production. Cell monolayers were infected with *S. neurona* merozoites, SN6 strain, isolated from a horse with EPM. Two hours after inoculation of the cells with merozoites, various concentrations of ponazuril were added to the media one time and the cultures allowed to incubate for 10 days. After 10 days of incubation, visual damage to the cell monolayer was assessed, media removed and quantified and the number of merozoites per mL determined by counting using a hemocytometer.

d. Results: Using a time of 10 days post-treatment, ponazuril inhibited merozoite production by more than 94% in bovine turbinate cultures of *S. neurona* treated with 0.1-1.0 µg/mL ponazuril and greater than 97% inhibition of merozoite production was observed when infected cultures were treated with 5 µg/mL. Results are shown in Table IV.1

Table IV.1 Percent reduction in merozoite production in cell cultures infected with *S. neurona* and treated with various concentrations of ponazuril

African green monkey cells

Ponazuril Concentration (µg/mL)	Merozoite count ±1sd	% Reduction
None	1,406,438 ±276,817	NA
0.1	412,062 ±102,227	70.7
1.0	139,125 ±200,878	90.1
5.0	40,125 ±43,758	97.1

Bovine turbinate cells

Ponazuril Concentration (µg/mL)	Merozoite count ±1sd	% Reduction
None	4,175,125 ±464,794	NA
0.01	3,040,688 ±410,257	27.2
0.1	231,813 ±93,988	94.4
1.0	63,313 ±46,594	98.5
5.0	86,375 ±58,853	97.9

e. **Conclusion:** Ponazuril is effective against the merozoite stage of *Sarcocystis neurona* in cell culture.

2. Pharmacokinetic Study

a. **Type of Study/Purpose:** Single phase multiple dose study with blood and cerebrospinal fluid samplings to determine the pharmacokinetics of ponazuril when given at 5 mg/kg as a 15% w/w oral paste formulation in normal horses. To determine the level of ponazuril in the serum and cerebrospinal fluid of horses prior to, during and after the administration of 5 mg/kg daily for 28 days.

b. **Investigator:**
Dr. Martin Furr, Marion duPont Scott Equine Center, Virginia Tech University, Old Waterford Rd. at Morven Park, P.O. Box 1938, Leesburg, VA 20177

c. **General Design:**

1) Animals: Ten healthy horses, all male castrates, ranging in age from 3-15 years

2) Treatment: Daily dose of 5 mg/kg (2.27 mg/lb) for 28 days with a 15% oral paste formulation of ponazuril

3) Treatment group assignment: Animals all received the same treatment.

4) Treatment Dose, Route, Frequency and Duration: Animals received ponazuril at a rate of 5 mg/kg body weight daily for 28 days.

5) Clinical Examination/Clinical Scoring: Animals were evaluated by physical examination, hematology and serum chemistry and cerebrospinal fluid indices.

6) Samples: Serum was collected for ponazuril assay at Days 0, 7, 14, 21, 28, 35, 36, 37, 38, 39, 40, 41, 42 and 49. Cerebrospinal fluid was collected for ponazuril assay at Days 0, 7, 14, 21, 28, 35, 42 and 49.

d. Results: Maximum calculated serum and CSF concentrations were 5.59 ug/mL and 0.21 ug/mL, respectively. These occurred on days 18.2 and 15 for serum and CSF, respectively. The terminal elimination half-life for serum (calculated using Day 28 to 42 data) was 4.50 ± 0.57 days. Steady state was achieved by approximately 20 days post dose, which is consistent with the terminal elimination half-life of approximately 4.5 days. The ratio of CSF to serum was approximately 4%.

The mean concentration vs. time profiles in serum and CSF are provided in Tables 1 and 2 below.

Table 1. Serum Concentrations (ug/mL) of Ponazuril, By Days

	0	7	14	21	28	35	36	37	38	39	40	41	42	49
Mean	0	4.33	5.26	5.18	4.66	0.394	0.289	0.236	0.2	0.167	0.152	0.124	0.106	0.101
SD	0	1.098	0.877	1.187	0.6	0.186	0.131	0.103	0.105	0.085	0.102	0.088	0.073	0.086

Table 2. CSF Concentrations (ug/mL) of Ponazuril, By Days

	0	7	14	21	28	35	42	49
Mean	0	0.182	0.156	0.169	0.156	0.02	0.001	0.001
SD	0	0.076	0.033	0.055	0.048	0.011	0.003	0.003

e. Conclusions: Ponazuril, when dosed at 5 mg/kg for 28 days, provides measurable drug concentrations in both serum and cerebrospinal fluid.

3. Summary of Dose Characterization: First, studies have demonstrated that ponazuril is an effective anticoccidial drug in several species of mammals and birds. Second, an *in vitro* evaluation has shown that ponazuril can significantly reduce *Sarcocystis neurona* merozoite production in cell cultures. Levels of drug in the range of 0.1-1.0 ug/mL were 94% effective when tested in bovine turbinate cell cultures and 70.7% effective when tested in African Green monkey cells. Third, ponazuril, when dosed to healthy horses at 5 mg/kg once daily for 28 days, provided mean daily steady state CSF concentrations ranging between 0.156 ug/mL to 0.182 ug/mL. In bovine cell culture this level was adequate to effect a 94% parasite kill rate.

B. Clinical Field Study 1

1. **Type of Study**: The study was conducted as a field evaluation of two doses of ponazuril (5 mg/kg and 10 mg/kg). The study was conducted by seven investigators involving a total of 102 acceptable cases.

2. **Investigators:**

Dr. Frank Andrews, Large Animal Clinical Medicine, College of Veterinary Medicine, University Of Tennessee, P.O. Box 1071, Knoxville, TN 37901-1701

Dr. Fairfield Bain, Hagyard-Davidson-Mcgee, 4250 Ironworks Road, Lexington, KY 40511-8412

Dr. Bill Bernard, Rood & Riddle Equine Hospital, 2150 Georgetown Road, Lexington, KY 40580

Dr. Doug Byars, Hagyard-Davidson-Mcgee, 4250 Ironworks Road, Lexington, KY 40511-8412

Dr. Martin Furr, Marion duPont Scott Equine Center, Virginia Tech University, Old Waterford Rd. at Morven Park, P.O. Box 1938, Leesburg, VA 20177

Dr. Robert Mackay, Dept. Large Animal Clinical Sciences, College of Veterinary Medicine, Box 100-136, University of Florida, Gainesville, FL 32610

Dr. Steven Reed, Dept. Large Animal Medicine, College of Veterinary Medicine, The Ohio State University, 611 Vernon L. Tharp Street, Columbus, OH 43210-6610

3. **General Design**: The use of historical controls in the evaluation of compounds for effectiveness is described in 21 CFR 514.117 (b)(4)(iv). Equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*, is a neurologic disease of horses that most often results in asymmetric incoordination (ataxia), weakness, and spasticity. EPM can occur as a peracute, acute, or chronic condition. The clinical signs are caused by direct neuronal damage by the parasite, as well as damage secondary to inflammation and the pressure exerted by inflammation within neural spaces. The evaluation of ponazuril as a potential anti-protozoal agent for the treatment of EPM was conducted using historical controls. Historical controls were used for this study due to the progressive nature of the disease if left untreated and the lack of approved therapeutics for the treatment of this disease. Effectiveness was based on a standardized neurologic grade scoring method with neurological examination corroborated with videotape reviews by an expert panel.

a. **Purpose**: This study was designed to evaluate the safety and effectiveness of 15% w/w ponazuril oral paste formulation when used according to label directions under field conditions in adult horses infected with *Sarcocystis neurona* and exhibiting neurological signs of equine protozoal myeloencephalitis (EPM).

b. **Animals**: There were one hundred two adult horses, 32 females, 9 males and 61 geldings, ranging in age from 2-30 years. Approximately 70% were Thoroughbreds

and Quarter Horses, with the remainder represented by Saddlebreds, Tennessee Walkers, Hanoverians, Appaloosas, Arabians and mixed breeds.

c. **Enrollment Criteria**: 2 years of age or older, weight appropriate for age and breed, acceptable overall physical condition score on physical examination, hematology, and serum chemistry values clinically acceptable, and a diagnosis of EPM supported by clinical examination and a positive Western Blot for *S. neurona* on CSF.

Primary Diagnostic Criterion - Standardized clinical examination consistent with EPM (presence of an asymmetric neurological deficit)

Neurologic Grading Scale:

0 – Normal, no deficit detected
1 – Deficit just detected at normal gait
2 – Deficit easily detected and is exaggerated by backing, turning, swaying, loin pressure or neck extension
3 – Deficit very prominent on walking, turning, loin pressure or neck extension
4 – Stumbling, tripping and falling down spontaneously
5 - Recumbent, unable to rise

Other enrollment criteria: cervical radiographs with no suggestion of spinal cord compression or vertebral canal stenosis (determined by calculation of adjusted minimal sagittal vertebral canal diameters and subjective assessment of radiographs), cerebrospinal fluid (CSF) positive Western blots for *Sarcocystis neurona* IgG, CSF cytology (less than 500 red blood cells/mL), CSF indices-(Total Protein <90, IgG index >0.3, albumin quotient <2.2), negative CSF for equine herpes virus-1 (EHV-1 titer below 1:4), and normal serum values for Vitamin E (> 2.0 µg/mL).

d. **Exclusion Criteria**: Animals that received therapy for EPM within the last 3 months, animals outside of the specifications above, animals with a questionable diagnosis (seizure disorders, behavioral disorders), any other clinical signs that may indicate diseases other than EPM.

e. **Treatment Groups and Controls**: Animals were randomly assigned to one of two treatment groups and dosed orally with either 5 mg/kg or 10 mg/kg of ponazuril in a 15% paste body weight daily for 28 days. The client did not know the dose. By study conclusion, 47 had been assigned to the 5 mg/kg dose and 55 to the 10 mg/kg dose.

f. **Challenge**: Natural infection.

g. **Dosage form**: The formulation used during this study was identical to the product intended for market, a 15% w/w ponazuril paste.

h. **Route of Administration**: Oral

i. **Dose, Frequency and Duration:** Animals were dosed at either 5 or 10 mg/kg body weight once per day for 28 days.

j. **Treatment Success or Failure**: Success was based on the clinical response and a masked scoring of videotapes made during the neurological examinations. Animals had to improve at least one grade on the neurological examination at 90 days after the

end of treatment, and had to be corroborated by video review to be considered a success.

4. **Results**: Of 113 horses evaluated, 11 were eliminated for non-compliance with the protocol, six at 5 mg/kg and five at 10 mg/kg. At a dose rate of 5 mg/kg (2.27 mg/lb.) for 28 days, 28 of 47 horses (60%) improved at least one grade by Day 118 based upon investigator's evaluation. At 10 mg/kg (4.54 mg/lb.) for 28 days, 32 of 55 animals (58%) improved at least one grade by Day 118 based upon investigator's evaluation.

   In order to corroborate the cases deemed successes by the clinical investigators, independent experts reviewed the videotapes of the success cases in a masked fashion. Of the 28 success cases at the 5 mg/kg dose, four horses did not have sufficient videotapes for review. Thus, of the remaining 24 success cases with videotapes, 18 were corroborated as successes (75% corroboration). Likewise, in the 10 mg/kg group, there were 32 success cases, 7 of which lacked videotapes. Thus of the remaining 25 cases with videotapes, 14 were corroborated as successes (56% corroboration).

   Because there was no difference between the 5 and 10 mg/kg doses with respect to the clinical investigators' assessments of success, the 5 mg/kg dose was selected.

EPM EFFECTIVENESS – USING CLINICAL INVESTIGATOR'S NEUROLOGICAL SCORES ONLY
DAY 0 EPM SCORES FROM CLINICAL INVESTIGATOR'S ASSESSMENT

Treatment	EPM SCORE D0 = 2	IMPROVED D118*	EPM SCORE D0 = 3	IMPROVED D118*	EPM SCORE D0 = 4	IMPROVED D118*
5 mg/kg **	28	15	15	10	4	3
% Improved	[redacted]	54%	[redacted]	67%	[redacted]	75%
10 mg/kg **	26	15	25	16	4	1
% Improved	[redacted]	58%	[redacted]	64%	[redacted]	25%

* based on clinical investigator's neurological assessment
** included all horses, whether videotaped or not

EPM EFFECTIVENESS – USING CORROBORATED VIDEOTAPE OUTCOMES
DAY 0 EPM SCORES FROM CLINICAL INVESTIGATOR'S ASSESSMENT

Treatment	EPM SCORE D0 = 2	IMPROVED D118*	EPM SCORE D0 = 3	IMPROVED D118*	EPM SCORE D0 = 4	IMPROVED D118*
5 mg/kg **	13	10	9	7	2	1
% Improved	[redacted]	77%	[redacted]	78%	[redacted]	50%
10 mg/kg **	12	5	12	8	1	1
% Improved	[redacted]	42%	[redacted]	67%	[redacted]	100%

* based on corroborated video outcomes
** did not include horses that were classified as successes by the clinical investigator but were missing a videotaped examination (N = 4 for 5 mg/kg group and N = 7 for 10 mg/kg group)

5. **Statistical Analysis**: Twenty-eight out of 47 (60%) and 32 out of 55 (58%) animals received a lower maximum score by the clinical investigator at three month's post-treatment when compared with pretreatment in the 5 and 10 mg/kg dose groups, respectively. There was no significant difference in the proportion of animals that showed improvement when comparing the two dose groups (Pearson's Chi-square, p=0.8867).

   No statistical analyses were performed on the CSF Western Blot data. All animals were found Western Blot CSF positive by at least one of two laboratories at the beginning of the study. The Western Blot of the CSF did not appear to be a major factor in determining treatment success nor a reliable measure of treatment success. The neurological examination (corroborated with masked videotape reviews) appeared to be the most reliable source for determining treatment success.

   Descriptive statistics were calculated for serum and cerebrospinal fluid variables. For serum IgG, 27.5% and 31.9% of the animals experienced an increase in the serum IgG Index in the 5 mg/kg and 10 mg/kg dose groups, respectively. The CSF IgG levels

d. **Exclusion criteria**: Animals outside of the specifications above, animals with a questionable diagnosis (seizure disorders, behavioral disorders), any other clinical signs that may indicate diseases other than EPM.

e. **Treatment Groups and Controls**: Animals were randomly assigned to one of two treatment groups and dosed orally with either 5 mg/kg or 10 mg/kg of ponazuril in a 15% w/w paste daily for 28 days. The client did not know the dose. By study conclusion, 7 horses had been assigned to the 5 mg/kg dose and 5 horses to the 10 mg/kg dose.

f. **Challenge**: Natural infection.

g. **Dosage form**: The formulation used during this study was identical to the product intended for market, a 15% w/w ponazuril paste.

h. **Route of Administration**: Oral

i. **Dose, Frequency and Duration**: Animals were dosed at either 5 or 10 mg/kg body weight once per day for 28 days.

j. **Treatment Success or Failure**: Success was based on the clinical response. Animals had to improve at least one grade on the neurological examination at the end of the 28 day treatment period to be considered a success.

4. **Results**: At a dose rate of 5 mg/kg (2.27 mg/lb.) for 28 days, 7 of 7 horses (100%) improved by Day 28. At 10 mg/kg (4.54 mg/lb.) for 28 days, 5 of 5 animals (100%) improved by Day 28.

TREATMENT	EPM SCORE D0 = 2	IMPROVED D28	EPM SCORE D0 = 3	IMPROVED D28	EPM SCORE D0 = 4	IMPROVED D28
5 mg/kg	2	2	4	4	1	1
% Improved		100		100		100
10 mg/kg	4	4	0		1	1
% Improved		100				100

5. **Statistics**: Due to the small number of animals in this study, no statistical analyses were completed.

6. **Conclusions**: Animals exhibiting signalment of equine protozoal myeloencephalitis that were treated with ponazuril at a dose of 5 or 10 mg/kg for 28 days showed improvement in clinical score of at least one grade in 100% of the cases in this study.

7. **Adverse Reactions**: There were no adverse reactions recorded during the study.

V. ANIMAL SAFETY

A. General Safety Study

1. **Type of Study**: Target Animal Safety- Toxicity Study

2. **Investigator**
John W. Campbell, Ph.D.
Southwest Bio-Labs, Inc.
Las Cruces, NM

3. **General Design**

a. **Purpose**: This study was designed to evaluate the safety of 15% w/w ponazuril oral paste formulation when given in multiple doses in the adult horse.

b. **Test Animals**: Sixteen adult mixed breed horses, 8 males and 8 females, ranging in age from 3-15 years.

c. **Control Animals**: Eight adult mixed breed horses, 4 males and 4 females, ranging in age from 3-15 years.

d. **Physical condition**: All animals were evaluated and found to be physically sound prior to the start of the study.

e. **Dosage form**: The formulation used during this study was identical to the product intended for market, a 15% w/w ponazuril paste.

f. **Route of Administration**: Oral

g. **Dosage Used**: Animals were dosed with ponazuril paste at 0 mg/kg, 10 mg/kg or 30 mg/kg body weight daily for 28 or 56 days.

h. **Variables Measured**: During the treatment phase, body weights were taken on Study Days 0, 7, 14, 21, 27, 42, 49, and 55, whole blood was collected for hematology, coagulation, and serum chemistry profiles on Study Days 7, 14, 21, 27 (all animals) and Days 35, 42, 49 and 55 for the horses treated for 56 days. Complete physical examinations were conducted on Study Days 27 (for all animals) and 55 for those horses treated for 56 days. Horses were observed once per week by a licensed veterinarian, and daily observations of the horses' general well being were made by the animal caretaker. Those making observations of the horses' physical condition were masked to treatment group. On Study Day 28, two pre-selected animals of each sex and from each group (12 total) were euthanized by an injection of euthanasia solution followed by exsanguination. A complete post-mortem examination was performed and all organ systems were examined and abnormalities were noted. Tissues were collected and prepared for histopathological examination by placing them into 10% buffered formalin. The eyes were injected with, then immersed in Davidson's fixative. A bone marrow smear was also prepared. The fixed tissues were sectioned and mounted, then routinely stained with hematoxylin and eosin and examined. The remaining 12 animals were euthanized and examined as above on Study Day 56.

4. **Statistical Analysis:**

Two analyses were conducted for each analysis endpoint. The first analysis included data from all 24 animals (8 animals/group) from day 0 through day 28 (Day 28 Analysis). The second analysis included data from day 0 through day 56 for the 12 animals (4 animals/group) that were treated for 56 days (Day 56 Analysis).

Each clinical pathology parameter was analyzed with a repeated measures analysis of covariance including terms for fixed effects, random effects and pre-treatment measurements as the covariate. The covariance structure was modeled as autoregressive order 1. Body weights were analyzed using the same model as described for clinical pathology with the day –1 measurement included as the covariate and the covariance structure modeled as compound symmetric. All tests were conducted at the 0.10 significance level.

During the study time period of 28 days, the analysis indicated that direct bilirubin was statistically significantly higher in both the 10 mg/kg and 30 mg/kg doses (p=0.094 and p=0.0542, respectively); white blood cell counts were statistically significantly higher in the 30 mg/kg dose (p=0.0016) and basophils were statistically significantly lower in the 30 mg/kg dose (p=0.0690).

In addition, at day 7, sodium was statistically significantly lower in the 30 mg/kg dose (p=0.0935); alanine aminotransferase was statistically significantly lower in both the 10 mg/kg and 30 mg/kg doses (p=0.0521 and p=0.0143, respectively); mean corpuscular volume was statistically significantly lower in both the 10 mg/kg and 30 mg/kg doses (p=0.0008 and p<0.0001, respectively); and mean corpuscular hemoglobin concentration was statistically significantly higher in the 10 mg/kg and 30 mg/kg doses (p=0.0195 and p=0.0015, respectively). At day 14, gamma glutamyltransferase was statistically significantly higher in the 30 mg/kg dose (p=0.0306); mean corpuscular volume was statistically significantly lower in both the 10 mg/kg and 30 mg/kg doses (p=0.084 and p=0.0053, respectively); and mean corpuscular hemoglobin concentration was statistically significantly higher in the 30 mg/kg dose (p=0.0016). At days 21 and 28, blood urea nitrogen was statistically significantly higher in the 30 mg/kg dose (p=0.0404 and p=0.0385, respectively).

During the study time period of 56 days, the analysis showed that chloride was statistically significantly higher in both the 10 mg/kg and 30 mg/kg doses (p=0.0431 and p=0.0837, respectively); total bilirubin was statistically significantly higher in the 30 mg/kg dose (p=0.0664); alanine aminotransferase was statistically significantly lower in the 30 mg/kg dose (p=0.0505); phosphate and gamma glutamyltransferase were statistically significantly higher in the 10 mg/kg dose (p=0.0017 and p=0.0649, respectively).

In addition, at day 7, mean corpuscular volume was statistically significantly lower in both the 10 mg/kg and 30 mg/kg doses (p=0.0838 and p=0.0263, respectively). At day 14, mean corpuscular hemoglobin was statistically significantly lower in the 10 mg/kg dose (p=0.0677); and mean corpuscular hemoglobin concentration was statistically significantly higher in the 30 mg/kg dose (p=0.0572). At day 28, mean corpuscular hemoglobin was statistically significantly lower in the 30 mg/kg dose (p=0.0044). At day 35, potassium was statistically significantly higher in the 10 mg/kg dose (p=0.0805).

For inappetence data, since only a few animals (4 animals) showed inappetence, there was no need to conduct statistical analysis.

The analysis of fecal scores using a generalized linear mixed model showed that both the 10 mg/kg and 30 mg/kg dose groups had statistically significantly higher abnormal

feces rates than the control group (p=0.0049 and p=0.0613, respectively) over the study time period of days 0-28; and only the 30 mg/kg dose group had statistically significantly higher abnormal feces rate than the control (p=0.0799) over the study time period of days 0-56.

The analysis of contingency tables summarizing fecal scores at each observation day using Fisher's Exact test indicated that a treatment effect was found for study days 5,6,15,19,20, and 39 ($p \leq 0.1$). For the pair-wise comparison between the control group and the 10 mg/kg group, statistically significant differences were shown for study days 5,6, and 20 ($p \leq 0.1$). Similarly, study days 24 and 25 exhibited statistically significant differences between the control group and the 30 mg/kg group ($p \leq 0.1$).

Body weight analyses resulted in no statistically significant effects for either study time periods of 28 or 56 days.

5. Results

a. Clinical Results-Hematology/Serum Chemistry: Sporadic findings outside of normal ranges were found in some animals. Any values noted as significantly different between groups were then subjected to further scrutiny to determine if a pattern or syndrome was evident. When values were noted as significantly different among groups and the values were noted consistently, the values were then compared to a table of standard values from the testing laboratory. The results of this evaluation showed the following:

1) Sodium (NA): This value is transient on day 7 and is not significant on any day after that. This transient hyponatremia may be due to increased water intake or increased sodium loss through the kidneys or manure. Values were not out of the normal range.

2) Direct Bilirubin (DBIL): The increased direct bilirubin concentration was not out of the normal range. Increased DBIL may be associated with mild biliary stasis and is related to increased GGT concentration on day 14, which is when the DBIL is the highest.

3) Blood Urea Nitrogen (BUN): There was a mild increase in BUN but not outside the normal range. Increased BUN would suggest increased metabolism of proteins or increased non-protein nitrogen absorption from the GI tract. The change in BUN was not associated with renal disease or decreased renal function. Serum creatinine, a more sensitive indicator of reduction in renal function, was not abnormal.

4) Potassium (K): There was a transient increase in potassium by day 35 but still within normal range. This value is actually normal but the control horses showed a decreased potassium level on that day. It would seem that the controls had a spuriously low concentration of potassium and potassium stayed low in controls throughout the study.

5) Chloride (Cl): There was an increased chloride concentration over time in treated horses, although within normal ranges for chloride. The change was not related to

dehydration, as NA does not increase with time, thus most likely it is related to decreased loss from kidneys.

6) Total Bilirubin (TBIL): Mild increase in total bilirubin may be related to mild biliary stasis as noted above.

7) White Blood Cells (WBC): There was a transient increase on Day 14. These values are still within the normal range. Increased WBC may be due to alteration in GI flora.

8) Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH): There was a transient decrease in RBC size, but still within the normal range which is related to the increase in MCHC. There is no known mechanism that increases MCHC over the normal range. The smaller RBCs are probably the result of older RBCs entering the circulation. This change is not associated with anemia, otherwise cells would be getting bigger or show an increased MCV.

9) Basophils (BASO): Basophils decreased over time but were within the normal range. These cells are a small number of the normal WBCs, can be quite variable, and usually increase with allergic reactions. The decrease would not be associated with allergic reaction in this case.

10) Gamma Glutamyltransferase (GGT): Increase in GGT over time suggests biliary stasis when noted with bilirubin changes. However, the change noted here was transient and of no clinical significance.

11) Phosphate (PO4): The increased phosphate noted in the 10 mg/kg group was within normal limits.

b. Clinical Results- Gastrointestinal-

1) Loose Feces: The incidence of loose feces, while noted in all groups including the controls, was greater in the 30 mg/kg group compared to controls.

2) Inappetence: Only four animals showed inappetence for some days during the study period. One animal was in the control group, two were in the 10 mg/kg dose group and one was in the 30 mg/kg dose group. Inappetance does not appear to be treatment related.

c. Body Weights: No statistical differences were noted in body weights among groups. However, one animal in the 10 mg/kg group lost weight over the course of 56 days.

d. Histopathology: Necropsy revealed moderate edema in uterine tissues of three of the four females in the 30 mg/kg group (two after 28 days of treatment and one after 56 days of treatment). The edema appeared to be treatment related.

6. Conclusions: Administration of ponazuril at a dose of 10 or 30 mg/kg body weight for 28 or 56 days produced transient episodes of loose feces as well as changes in uterine tissues at 30 mg/kg. Overall, ponazuril administered as a 15% oral paste, at 2X or 6X the recommended dosage, was demonstrated to be generally safe for adult horses.

VI. HUMAN SAFETY

Data on human safety, pertaining to consumption of drug residues in food, were not required for approval of this NADA. The drug is to be labeled for use in horses, which are non-food animals.

Human warnings are provided on the product label as follows:

WARNING: For use in animals only. Not for use in horses intended for food. Not for human use. Keep out of the reach of children.

VII. AGENCY CONCLUSIONS:

The data in support of this NADA comply with the requirements of Section 512 of the Act and Section 514 of the implementing regulations. The data demonstrate that Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste, when used under labeled conditions of use, is safe and effective.

The drug is restricted to use by or on the order of a licensed veterinarian because professional expertise is required to diagnose EPM and to monitor the safe use of this product.

Under Section 512(c)(2)(F)(i) of the Federal Food, Drug, and Cosmetic Act, this approval qualifies for FIVE years of marketing exclusivity beginning on the date of approval because no active ingredient (including any ester or salt of the active ingredient) has been approved in any other application.

Bayer Animal Health, Inc. has the following patent: U.S. Patent No. 5,833,095 which expires on August 7, 2017.

VIII. APPPROVED PRODUCT LABELING

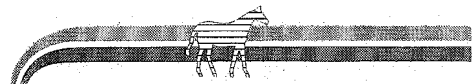
1. Package Insert: Side 1 & 2
2. 127g Syringe Label
3. Carton for 4 x 127g Syringe Applicators plus Reusable Plunger: Front Panel, Side Panel, Back Panel, Top Flap, Inside Top

Marquis™

(15% w/w ponazuril)

Antiprotozoal Oral Paste

Caution: Federal (U.S.A.) Law restricts this drug to use by or on the order of a licensed veterinarian.



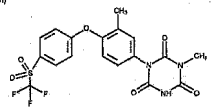
For The Treatment Of Equine Protozoal Myeloencephalitis (EPM) In Horses
Oral Use Only

DESCRIPTION: Marquis (15% w/w ponazuril) Antiprotozoal Oral Paste is supplied in ready-to-use syringes containing 127 grams of paste. Each gram of paste contains 150 mg of ponazuril (15% w/w). Marquis (ponazuril) is designed to be delivered as an orally administered paste.

Each syringe barrel of Marquis (ponazuril) contains enough paste to treat one (1) 1,200 lb (544 kg) horse for seven (7) days, at a dose rate of 5 mg/kg (2.27 mg/lb) body weight. The plunger contains a dosage ring calibrated for a dose rate of 5 mg/kg (2.27 mg/lb) body weight and marked for horse weight from 600 to 1,200 lbs (272 to 544 kg). The syringe barrels are packaged in units of four with one reusable plunger. This package provides sufficient paste to treat one 1,200 lb (544 kg) horse for 28 days at a dose rate of 5 mg/kg (2.27 mg/lb) body weight.

Ponazuril is an anticoccidial (antiprotozoal) compound with activity against several genera of the phylum Apicomplexa.

CHEMICAL NOMENCLATURE AND STRUCTURE: Ponazuril
1,3,5-Triazine-2,4,6(1H, 3H, 5H)-trione,1-methyl-3-[3-methyl-4-[4-[(trifluoromethyl)sulfonyl] phenoxy]phenyl]-(9CI)



CLINICAL PHARMACOLOGY: The activity of ponazuril has been demonstrated in several Apicomplexans[1-4]. Lindsay, Dubey and Kennedy[1] showed that the concentration of ponazuril necessary to kill *Sarcocystis neurona in vitro* was 0.1 to 1.0 µg/mL. Furr and Kennedy[4] evaluated the pharmacokinetics of ponazuril in serum and CSF in normal horses treated daily at 5 mg/kg for 28 days. The time to peak serum concentration (T_{max}) was 18.20 (±5.9) days and the maximum serum concentration (C_{max}) was 5.59 (±0.30) µg/mL. The terminal elimination half-life for serum (calculated using Day 28 to 42 data) was 4.50 (±0.57) days. In CSF, T_{max} was 15.40 (±7.9) days and C_{max} was 0.21 (±0.072) µg/mL.

INDICATIONS: Marquis (ponazuril) is indicated for the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*.

EFFECTIVENESS SUMMARY: A field study was conducted at six sites with seven investigators across the United States.[4] The study was conducted using historical controls. In this study, each animal's response to treatment was compared to its pre-treatment values. The following standardized neurologic scale was used to grade the horses:

0 – Normal, no deficit detected
1 – Deficit just detected at normal gait
2 – Deficit easily detected and is exaggerated by backing, turning swaying, loin pressure or neck extension
3 – Deficit very prominent on walking, turning, loin pressure or neck extension
4 – Stumbling, tripping and falling down spontaneously
5 – Recumbent, unable to rise

Improvement was defined as a decrease of at least one grade.

Naturally-occurring clinical cases of EPM, characterized by signalment and laboratory diagnosis, were randomly allotted to one of two treatment doses (5 or 10 mg/kg/day for a period of 28 days), then evaluated for clinical changes through 118 days. Acceptance into the study was based on the results from a standardized neurological examination including radiography, serum *S. neurona* IgG level determination by Western Blot (WB), and a positive cerebrospinal fluid (CSF) for *S. neurona* IgG level by WB.

Response to treatment was determined by the investigator to be acceptable when a clinical improvement of at least one grade occurred by no later than 3 months after treatment, regardless of whether the CSF by WB was positive or negative.

Changes in clinical condition were evaluated first by the subjective scoring of the investigator, then by masked assessment of videotapes of the neurological examination. At 5 mg/kg for 28 days, 28 of 47 horses (60%) improved at least one grade by Day 118. Seventy-five percent (75%) of those improved, that had also been videotaped, were corroborated successes by videotape assessment. At 10 mg/kg, 32 of 55 animals (58%) improved at least one grade by Day 118 and 55% of those improved, that had also been videotaped, were corroborated successes using videotape assessment. With respect to the clinical investigator's scores there was no statistical difference between 5 mg/kg and 10 mg/kg treatment group results (p = 0.8667).

WARNING: For use in animals only. Not for use in horses intended for food. Not for human use. Keep out of the reach of children.

PRECAUTIONS: Prior to treatment, EPM should be distinguished from other diseases that may cause ataxia in horses. Injuries or lameness may also complicate the evaluation of an animal with EPM. In most instances, ataxia due to EPM is asymmetrical and affects the hind limbs.

Clinicians should recognize that clearance of the parasite by ponazuril may not completely resolve the clinical signs attributed to the natural progression of the disease. The prognosis for animals treated for EPM may be dependent upon the severity of disease and the duration of the infection prior to treatment.

The safe use of Marquis (ponazuril) in horses used for breeding purposes, during pregnancy, or in lactating mares has not been evaluated. The safety of Marquis (ponazuril) with concomitant therapies in horses has not been evaluated.

ADVERSE REACTIONS: In the field study, eight animals were noted to have unusual daily observations. Two horses exhibited blisters on the nose and mouth at some point in the field study, three animals showed a skin rash or hives for up to 18 days, one animal had loose stools throughout the treatment period, one had a mild colic on one day and one animal had a seizure while on medication. The association of these reactions to treatment was not established.

ANIMAL SAFETY SUMMARY: Marquis (ponazuril) was administered to 24 adult horses (12 males and 12 females) in a target animal safety study. Three groups of 8 horses each received 0, 10, or 30 mg/kg (water as control, 2X and 6X for a 5 mg/kg [2.27 mg/lb] dose). Horses were dosed after feeding. One half of each group was treated for 28 days and the other half for 56 days followed by necropsy upon termination of treatment. There were several instances of loose feces in all animals in the study irrespective of treatment, sporadic inappetence and one horse at 10 mg/kg (2X) lost weight while on test. Loose feces were treatment related. Histopathological findings included moderate edema in the uterine epithelium of three of the four females in the 6X group (two treated for 28 days and one for 56 days).

For a copy of the Material Safety Data Sheet (MSDS) or to report Adverse Reactions, call Bayer Customer Service at (800) 633-3796.

Marquis™
(15% w/w ponazuril)
Antiprotozoal Oral Paste

79004570, R.1

8826

Bayer

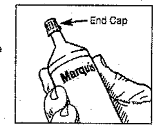
Manufactured by Bayer Corporation, Agriculture Division, Animal Health, Shawnee Mission, Kansas 66201 U.S.A.

DOSAGE: Marquis (ponazuril) is to be used at a dose of 5 mg/kg (2.27 mg/lb) body weight once daily for a period of 28 days.

ADMINISTRATION:

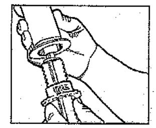
Paste syringe assembly:

Before administration, the syringe barrel and plunger require assembly. Ensure plunger is clean and dry.



Step 1. End cap **must** be on syringe barrel when inserting plunger.

Step 2. Carefully insert reusable plunger into base of syringe barrel until it snaps into place, then remove end cap and gently apply pressure to the plunger until paste is seen at the tip of the syringe barrel.



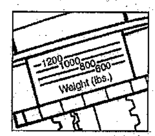
Step 3. Return end cap to tip of paste syringe.

Administering Marquis (ponazuril) to the horse:

NOTE: The paste syringe is a multi-dose package. Ensure that the correct dose is administered with each use.

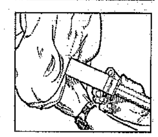
Step 1. Remove end cap and gently apply pressure to the plunger until paste is seen at the tip of the syringe barrel. Return end cap to tip of paste syringe.

Step 2. Determine weight of horse and ensure the horse's mouth contains no feed.



Step 3. To measure dose, dosage ring collar and barrel collar should be flush. Hold plunger and rotate dosage ring with the other hand to the weight of the horse.

Step 4. Remove end cap from tip of syringe barrel.



Step 5. The selected dose of paste should be deposited onto the back and top of the horse's tongue. Introduce tip of paste syringe into the side of the horse's mouth at the space between the front (incisor) and back (molar) teeth. Deposit paste on the horse's tongue by depressing the plunger of the syringe as far as the dose ring permits. Remove tip of syringe from horse's mouth.

Step 6. To aid swallowing of paste, immediately raise horse's head for a few seconds after dosing.

Step 7. Clean the tip of the syringe with a clean disposable towel and return end cap to tip of syringe barrel.

Step 8. For the next daily dose, repeat steps 1-7.

NOTE: ***When the paste syringe barrel is empty, remove plunger for re-use and assembly with a new syringe barrel.*** *When removed, the plunger may retain a seal from the empty paste syringe barrel. If this occurs, remove the seal before plunger is inserted into the base of the new paste syringe barrel. At the end of the prescribed treatment period, partially used syringes should be discarded.*

STORAGE: Store at Controlled Room Temperature 15-30° C (59-86° F).

HOW SUPPLIED:

Code: 045799 Carton contains four (4) x 127 gram syringe applicators and one (1) reusable syringe plunger

REFERENCES:

1. Mehlhorn, H., Ortmann-Falkenstein, G., Haberkorn, A.: (1984) The effects of the sym. Trianzinone on developmental stages of *Eimeria tenella, E. maxima* and *E. acervulina:* a light and electron microscopical study. Zeitschr Parsitenk 70: 173-182.
2. Bohrmann, R.: (1991) Treatment with toltrazuril in a natural outbreak of coccidiosis in calves. Dtsch. Tierarzl. Wschr. 98: 343-345.
3. Stafford, K.J., West, D.M., Vermunt, J.J., Pomroy, W., Adlington, B.A., Calder, S.M.: (1994) The effect of repeated doses of toltrazuril on coccidial oocyst output and weight gain in infected lambs. NZ Vet J 42(3): 117-119.
4. Haberkorn, A.G., Stoltefuss, D.I.J.: (1987) Studies on the activity spectrum of toltrazuril, a new anti-coccidial compound. Vet. Med. Review 1: 22-32.
5. Koudela, B., Vodstrcilova, M., Klimes, B., Vladik, P., Vitovec, J.: (1991) Application of the anticoccidiosis drug Toltrazuril in the coccidiosis of neonatal pigs. Veterinarni medicina (Praha) 36: 657-663.
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7. Lindsay, D.S., Dubey, J.P., Kennedy, T.J.: (2000) Determination of the activity of ponazuril against *Sarcocystis neurona* in cell cultures. Vet Parasit 92: 165-169.
8. Furr, M., Kennedy, T.: Pharmacokinetics of ponazuril in horses. Study 150-717 Bayer Corporation.
9. Furr, M., Andrews, F., MacKay, R., Reed, S., Bernard, W., Bain, F., Byars, D., Kennedy, T.: Treatment of equine protozoal myeloencephalitis (EPM) with various doses of ponazuril. Study Report 150-664 Bayer Corporation.

U.S. Patent No. 5,833,095

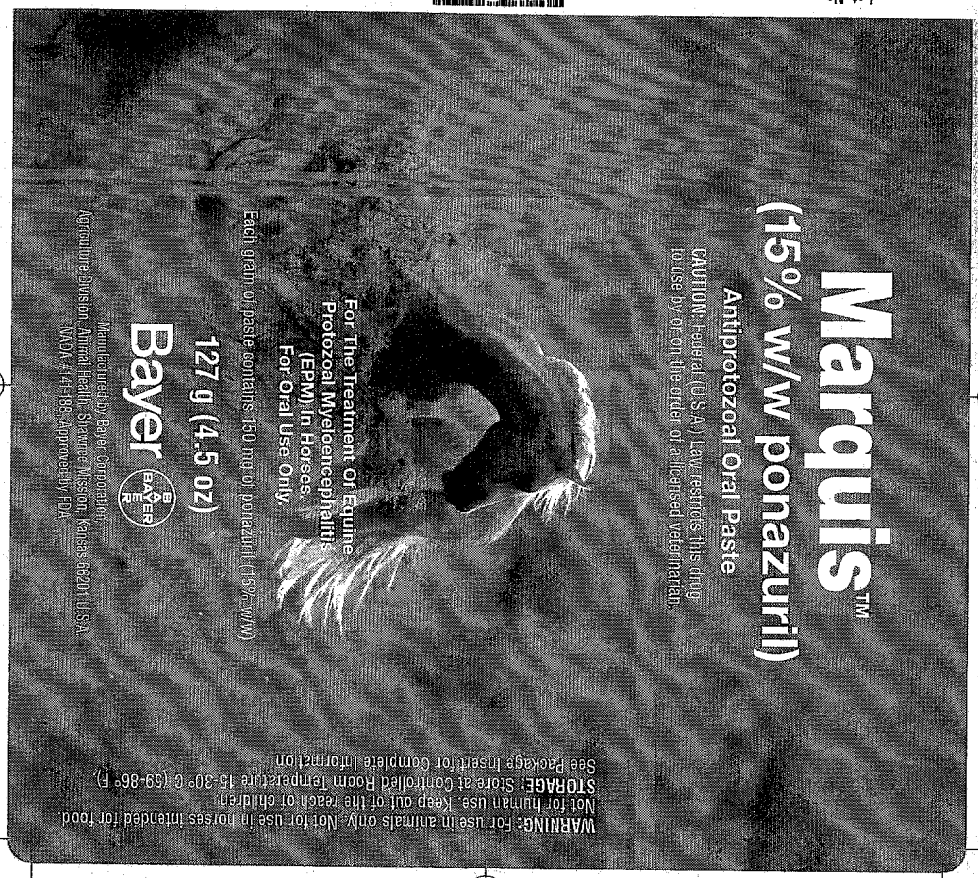
79004570, R.1
July, 2001

Manufactured by Bayer Corporation,
Agriculture Division, Animal Health,
Shawnee Mission, Kansas 66201 U.S.A.

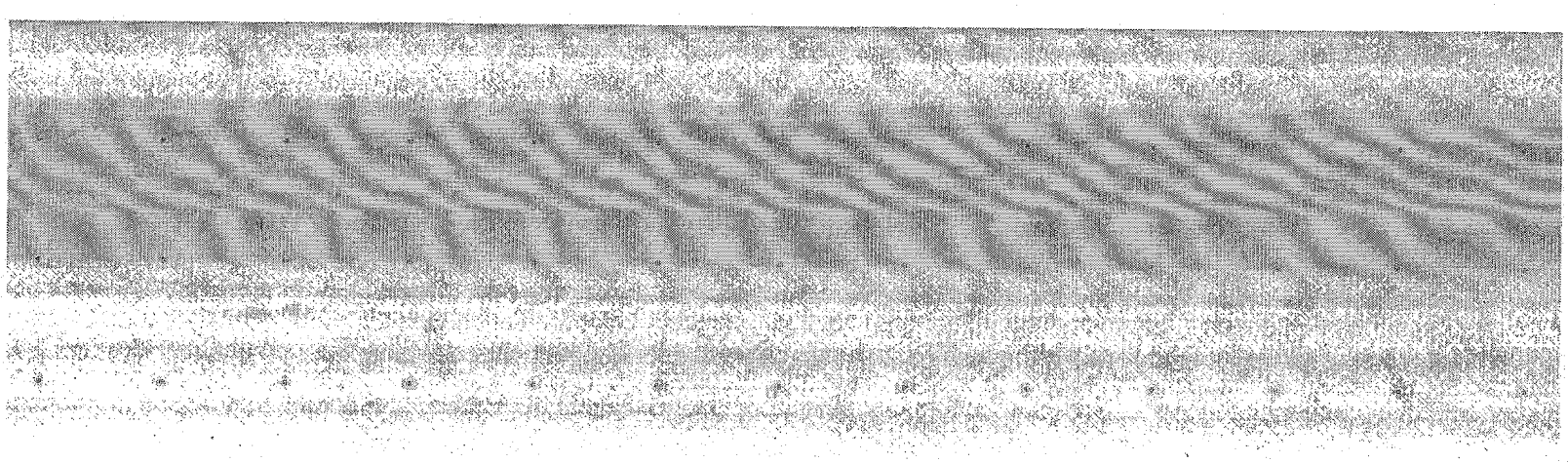
NADA #141-188, Approved by FDA

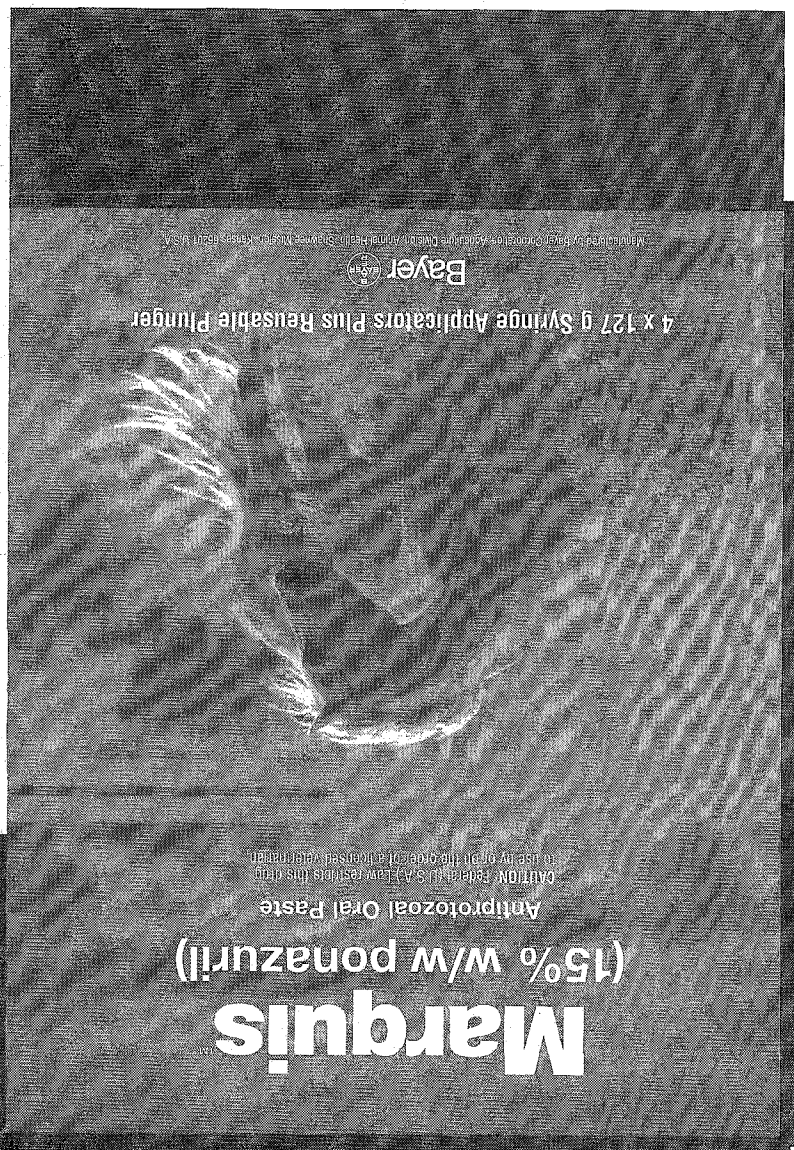
Bayer

Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste
NADA – 141-188
Package Insert – Side 2



Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste
NADA – 141-188
Syringe Unit Label





Marquis™
(15% w/w ponazuril)
Antiprotozoal Oral Paste

4 x 127 g Syringe Applicators
Plus Reusable Plunger

For The Treatment Of
Equine Protozoal Myeloencephalitis
(EPM) In Horses
For Oral Use Only

FPO

7 24089 90457 X

ADMINISTRATION:

Carton contents:

Exploded view of dosage ring

Paste s

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Step 1:
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gently apply pressure to
the plunger until paste is
seen at the tip of the
syringe barrel.

Step 3: Return and cap
tip of paste syringe.

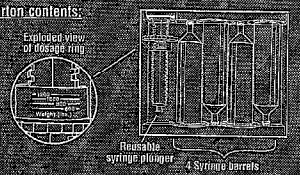
NOTE: When the paste s
When removed, the plu
before plunger is inserte
partially used syringes s

Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste
NADA – 141-138
Multi-Carton
Top Flap and Back Panel

Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste
NADA – 141-188
Multi-Carton
Inside Top Flap

ADMINISTRATION:

Carton contents:



Paste syringe assembly:

Before administration, the syringe barrel and plunger require assembly. Ensure plunger is clean and dry.

Step 1. End cap **must** be on syringe barrel when inserting plunger.

Step 2. Carefully insert reusable plunger into base of syringe barrel until it snaps into place, then remove end cap and gently apply pressure to the plunger until paste is seen at the tip of the syringe barrel.

Step 3. Return end cap to tip of paste syringe.

Administering Marquis (ponazuril) to the horse:

Note: The paste syringe is a multi-dose package. Ensure that the correct dose is administered with each use.

Step 1. Remove end cap and gently apply pressure to the plunger until paste is seen at the tip of the syringe barrel. Return end cap to tip of paste syringe.

Step 2. Determine weight of horse and insure the horse's mouth contains no feed.

Step 3. To measure dose, dosage ring collar and barrel collar should be flush. Hold plunger and rotate dosage ring with the other hand to the weight of the horse.

Step 4. Remove end cap from tip of syringe barrel.

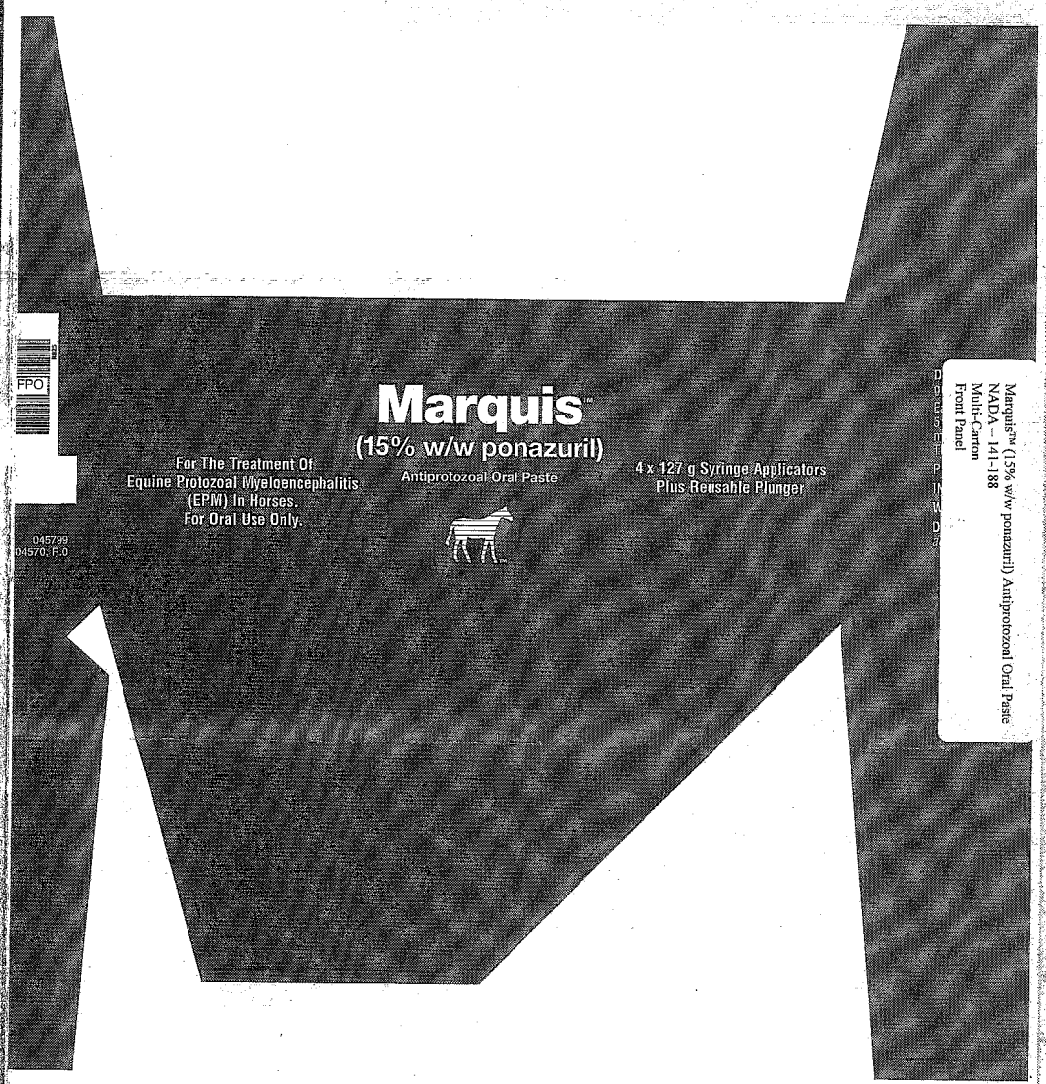
Step 5. The selected dose of paste should be deposited onto the back and top of the horse's tongue. Introduce tip of paste syringe into the side of the horse's mouth at the space between the front (incisor) and back (molar) teeth. Deposit paste on the horse's tongue by depressing the plunger of the syringe as far as the dose ring permits. Remove tip of syringe from horse's mouth.

Step 6. To aid swallowing of paste, immediately raise horse's head for a few seconds after dosing.

Step 7. Clean the tip of the syringe with a clean disposable towel and return end cap to the tip of the syringe barrel.

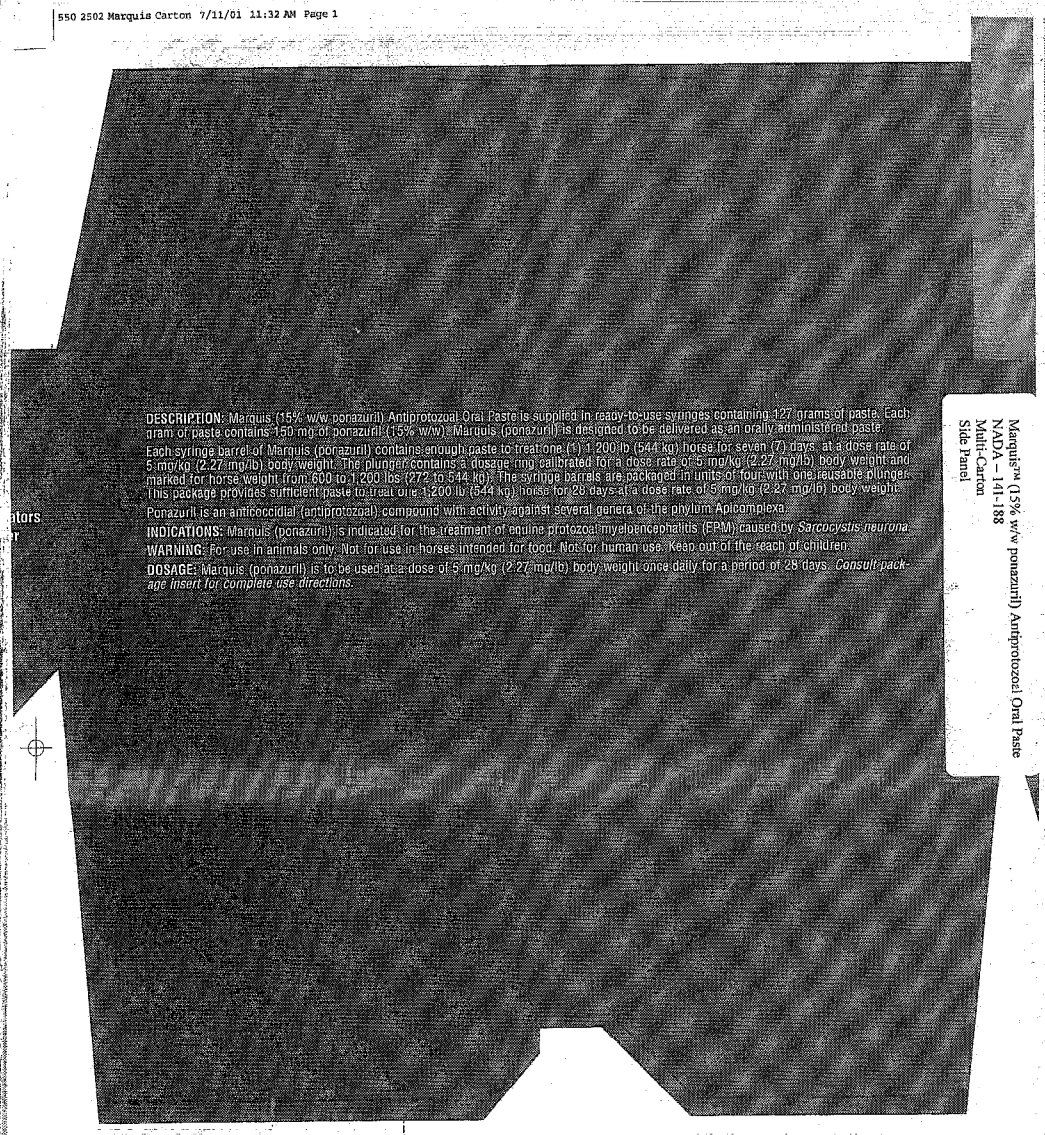
Step 8. For the next daily dose, repeat steps 1-7.

NOTE: *When the paste syringe barrel is empty,* ***remove plunger for re-use and assembly with a new syringe barrel.*** *When removed, the plunger may retain a seal from the empty paste syringe barrel. If this occurs, remove the seal before plunger is inserted into the base of the new paste syringe barrel. At the end of the prescribed treatment period, partially used syringes should be discarded.*



Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste
NADA – 141-188
Multi-Carton
Front Panel

Background Color: PMS 4625



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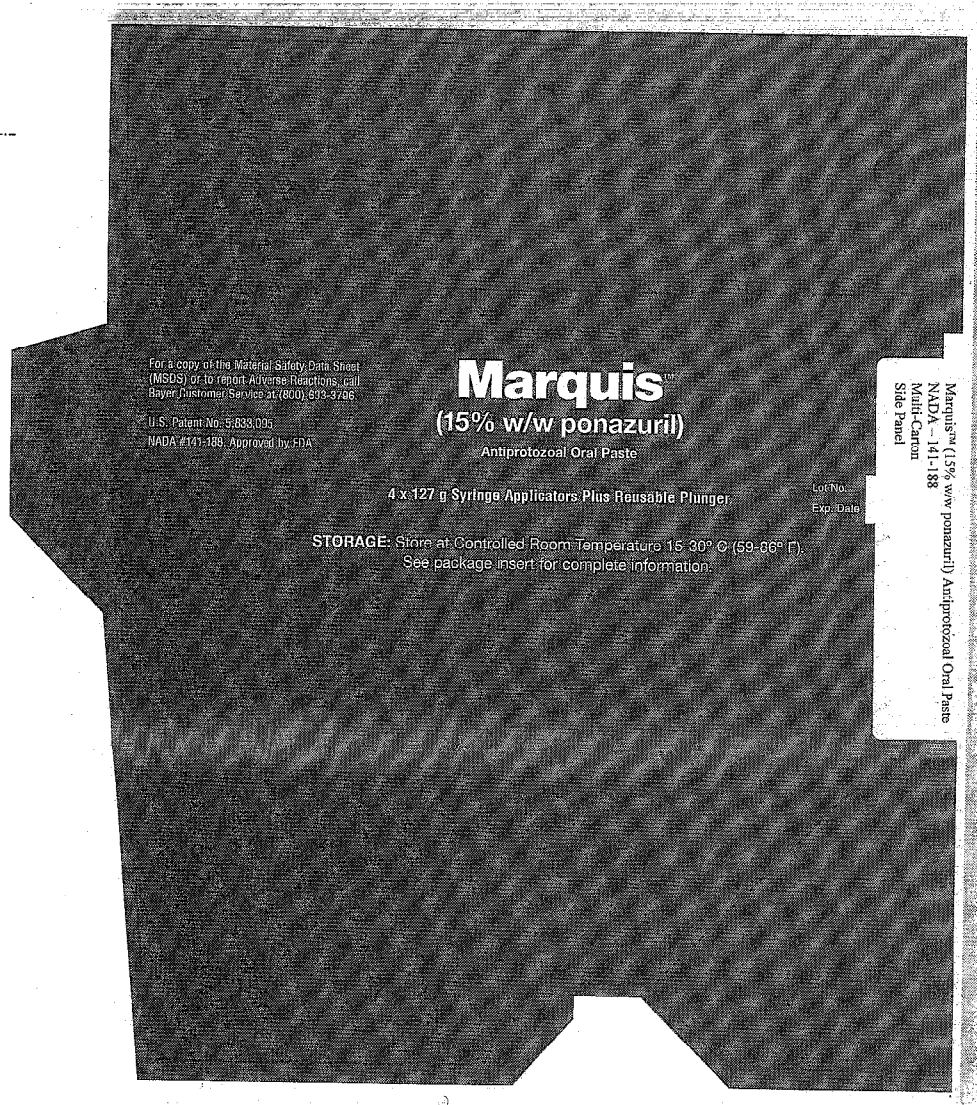
Ponazuril is an anticoccidial (antiprotozoal) compound with activity against several genera of the phylum Apicomplexa.

INDICATIONS: Marquis (ponazuril) is indicated for the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*.

WARNING: For use in animals only. Not for use in horses intended for food. Not for human use. Keep out of the reach of children.

DOSAGE: Marquis (ponazuril) is to be used at a dose of 5 mg/kg (2.27 mg/lb) body weight once daily for a period of 28 days. *Consult package insert for complete use directions.*

Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste
NADA – 141-188
Multi-Carton
Side Panel



Customer: Bayer Agriculture	Material: E-WHITE
Design: x10598	Description: 4 PACK TUBE AND PLUNGER
Size: 7+7/8 x 9+5/8 x 2+5/8	Side Shown: Printside
Style: Tuck Top Auto Bottom	Date: 04/05/2000

Marquis™ (15% w/w ponazuril) Antiprotozoal Oral Paste
NADA 141-188

Shipper Label

Marquis™

Code 0457

(15% w/w ponazuril)

Antiprotozoal Oral Paste

6 x 4 x 127g

Store at Controlled Room Temperature 15-30°C (59-86°F)

Bayer

Bayer Corporation
Agriculture Division, Animal Health
Shawnee Mission, Kansas 66201

Lot: 0000000
Exp: 000000

Made in the USA R.1.